Einladung zum Oberseminar Wissenschaftliches Rechnen

Julius-Maximilians-Universität Würzburg
Lehrstuhl für Wissenschaftliches Rechnen IX

Ort: Raum 30.02.003 (2. Stock) (Mathegeb. 30 West) Zeit: Dienstag, 15.01.2013, um 16.00 Uhr

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Real-time monitoring of receptor signalling in living cells: from intracellular signalling microdomains to single molecules

G-protein-coupled receptors (GPCRs) constitute the major family of cell surface receptors. They comprise receptors for light, taste and smell as well as ions, small transmitters, peptides and large protein hormones. Given their involvement in fundamental biological processes and their accessibility, GPCRs serve as targets for several drugs, including beta-blockers, antihistamines and opiates.

Whereas many biochemical steps involved in GPCR signalling have been characterised in good details, their spatio-temporal dynamics is still largely unknown. This is because biochemical techniques have limited temporal and, generally, no spatial resolution. In the last years, our group developed a series of optical methods based on fluorescence resonance energy transfer (FRET), which allow imaging GPCR activation and signalling in real-time in living cells. More recently, in order to analyse GPCR signalling under highly physiological conditions, we generated a transgenic mouse with ubiquitous expression of a FRET sensor for the intracellular second messenger cyclic AMP (cAMP) (1,2). This mouse allowed us among others to study the signals produced by the activation of a prototypical hormone receptor, i.e. the thyroid stimulating hormone receptor (TSHR), directly in intact thyroid follicles. Unexpectedly, the results indicate that the TSHR and possibly other GPCRs can continue stimulating cAMP production after internalisation into the endosomal compartment, leading to persistent signalling and specific effects (2,3). These data reveal new and important functions for receptor internalisation in regulating GPCR-mediated responses.

To further advance our knowledge of the spatiotemporal dynamics of GPCR signalling, we have recently developed additional methods based on labelling with small organic fluorophores and total internal reflection fluorescence (TIRF) microscopy, which allow visualising signalling proteins at the surface of living cells with single-molecule sensitivity.

We are using these methods to monitor individual protein-protein interactions such as those involved in ligand binding, receptor di-/oligomerisation or coupling to G-proteins with high spatiotemporal resolution. Initial data on GPCR di-/oligomerisation suggest that GPCRs are present on the cell surface in a dynamic equilibrium, with constant formation and dissociation of new receptor complexes, which differ in size among different receptors and can be targeted, in a ligand-regulated manner, to different membrane microdomains (4). Taken together, these data provide novel insights into the complex and dynamic events at the basis of the spatiotemporal compartmentalisation of GPCR signalling cascades.

Literatur

[1] Nikolaev VO et al. Novel single chain cAMP sensors for receptor-induced signal propagation. J Biol Chem 2004; 279:37215-8.

[2] Calebiro D et al. Persistent cAMP-signals triggered by internalized G-protein-coupled receptors. PLoS Biol 2009; 7:e1000172.

[3] Calebiro D et al. Signaling by internalized G-protein-coupled receptors. Trends Pharmacol Sci 2010; 31:221-8.

[4] Calebiro D et al. Single-molecule analysis of fluorescently labeled G-protein-coupled receptors reveals complexes with distinct dynamics and organization. Proc Natl Acad Sci U S A 2012 [Epub ahead of print].

Zu diesem Vortrag laden wir Sie herzlich ein.

gez. Prof. Dr. Alfio Borzi

gez. Prof. Dr. Bastian von Harrach